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A NEW THEORY ON THE MECHANICS OF CILIARY AND FLAGELLAR MOTILITY. II. THEORETICAL CONSIDERATIONS[1]

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Evidence presented in the preceding paper (Costello, 1973) strongly suggests certain characteristics of axonemal microtubules. (1) Doublet microtubules have an inherent tendency towards bending or coiling, the expression of this tendency being governed by the length of the axoneme. (2) Central singlet microtubules of the 9 + 2 configuration, or the core of the "9 + 1" pattern, in contrast, become stiff or straight under certain conditions. (3) Active bending of the doublets is in the direction away from their dynein arms, so that the B-subtubule is on the concave side. These observations may all be correlated into a unifying hypothesis to account for the mechanics of ciliary and flagellar motility.

An earlier theory, along somewhat the same lines, was that of Bradfield, (1955), who treated all axonemal components as single units. At that time, all that was known of the substructure of the peripheral "fibers" was that there were two "subfibers" per peripheral "fiber" in cilia and many flagella. However, even this fact was ignored by Bradfield in the formulation of his theory. A reconsideration of these older ideas is therefore highly desirable.

Theory

General aspects

The theory depends upon certain assumptions and corollaries, which follow:

(1) Both singlet and doublet axonemal microtubules can exist in either the activated or the relaxed state. In the activated state, the doublets bend, while the singlets straighten and stiffen, and the bending or straightening waves are propagated along the respective types of microtubules, at different rates and with differing durations.

(2) We assume that the resistance to bending of an axoneme due to stiffening of the central singlets is greatest in the plane connecting the axes of the singlets where they are fastened together and thus reinforce each other, and least in the direction at right angles to this plane. Partly because there are more doublets than singlets, we assume also that the active bending of three doublets in this latter direction is quite sufficient to overcome the resistance and produce basal bending of the stiffened singlets and of the intact cilium.

(3) In the relaxed condition, both types of microtubules may possess some degree of structural rigidity rather than being completely limp. In addition, they are held in normal association to each other by the matrix of the organelle, by the

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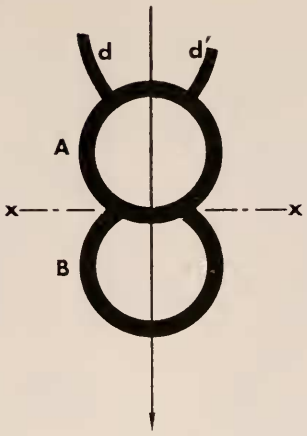


FIGURE 1. Diagram of doublet microtubule, showing direction of bending (arrow) at right angles to axis X--X; A = complete microtubule; B = incomplete tubule; d, d' = dynein arms.

"spokes" (radial connectives between doublets and central structures), by the connectives to the plasma membrane from the doublets, by the nexin links (circumferential connectives) between the A-subtubules (Stephens, 1970), and by the bridges between the singlets of the central pair, where these exist. In this relaxed state, non-activated microtubules of the axoneme can be moved passively in any direction by those doublet microtubules which become activated and bend.

(4) It is assumed that the waves propagated out the doublets are initiated successively, in unidirectional order, by impulses arising at or under the basal plate. The activated doublets bend in a direction dictated by their ultrastructural organization, namely, in a direction away from their dynein arms (Fig. 1).

(5) Activation of successive doublets, to bend in the directions contributing to either the effective or recovery strokes of a cilium or to the planar or helical movement of a flagellum, is responsible for these types of movement. However, the organelle as a whole moves with its characteristic wave pattern because of its length-wavelength-amplitude relationships and the coordination brought about through integrating these with the behavior of the singlets (or core complex of the "9 + 1" pattern), where these are present, and with the connectives of the axoneme listed above. The bending forces generated in the doublets must act against any normal structural resistance of the relaxed doublets, and against the stiffening forces produced by the central singlets (or the core), as well as against the resistance of the medium. This interaction, in flagella at least, should facilitate unbending, the mechanism of which has always been a problem (Brokaw, 1968; Brokaw, Goldstein and Miller, 1970).

Ciliary motility

This theory will first be applied to an "ideal" simple cilium. An "ideal" cilium is here defined as having an axonemal contour length sufficient for exactly $\frac{1}{2}$ wavelength of beat. A hypothetical commutator-type stimulator, located at or

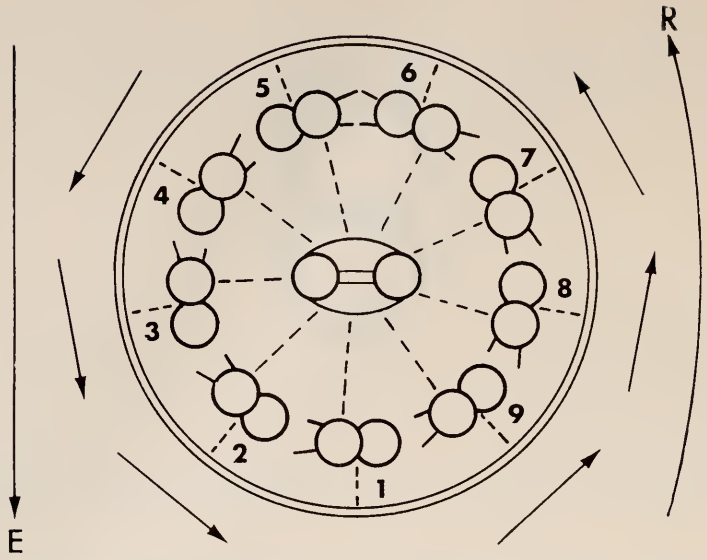


FIGURE 2. Diagram of cross-section of a cilium with doublets numbered #1 through #9; arrow E = direction of effective stroke; arrow R = recovery stroke.

under the basal plate, stimulates the basal end of doublet #1 (as conventionally numbered) to initiate an impulse which will be propagated up this doublet (Figs. 2 and 3E). It is suggested, also, that a stimulus is sent earlier or at the same time to each of the two central singlets, to initiate stiffening impulses which will move up these singlets at a considerably greater rate of propagation. The stimulation of doublet #1 is essentially ineffective in producing any bending in the direction away from its dynein arms because the stiffened singlets are so fastened together by the bridges between them that movement laterally is not possible. This resistance to lateral movement is reinforced by the arrangement of the ciliary rootlets. It is possible, also, that a bending wave moving up a single doublet is insufficient, in itself, to produce much, if any, bending of a cilium as a whole, especially in this direction. A reinforced bending is presumably very much more effective. However, any movement that is produced by doublet #1 would result only in a little basal bending and cause the stiff body of the cilium to incline very slightly to one side (toward doublet #9 of Fig. 2).

The wave stiffening the central singlets would need to travel about four and a half times as fast as the wave propagated to bend a peripheral doublet in order to stiffen the cilium all the way to its tip, by the time the commutator has shifted clockwise to activate doublet #2 to begin its basal bending. This is because of the axonemal length which is a function of $\frac{1}{2}$ the wavelength in the ideal cilium. As the hypothetical commutator travels on to trigger basal bending in doublet #3 and then in doublet #4, the central singlets must be presumed to remain stiff throughout their lengths. While doublets #2, #3 and #4 each bend in the direction away from their dynein arms, for the cilium as a whole the resultant of the bending forces is in the direction toward doublet #1 and away from the

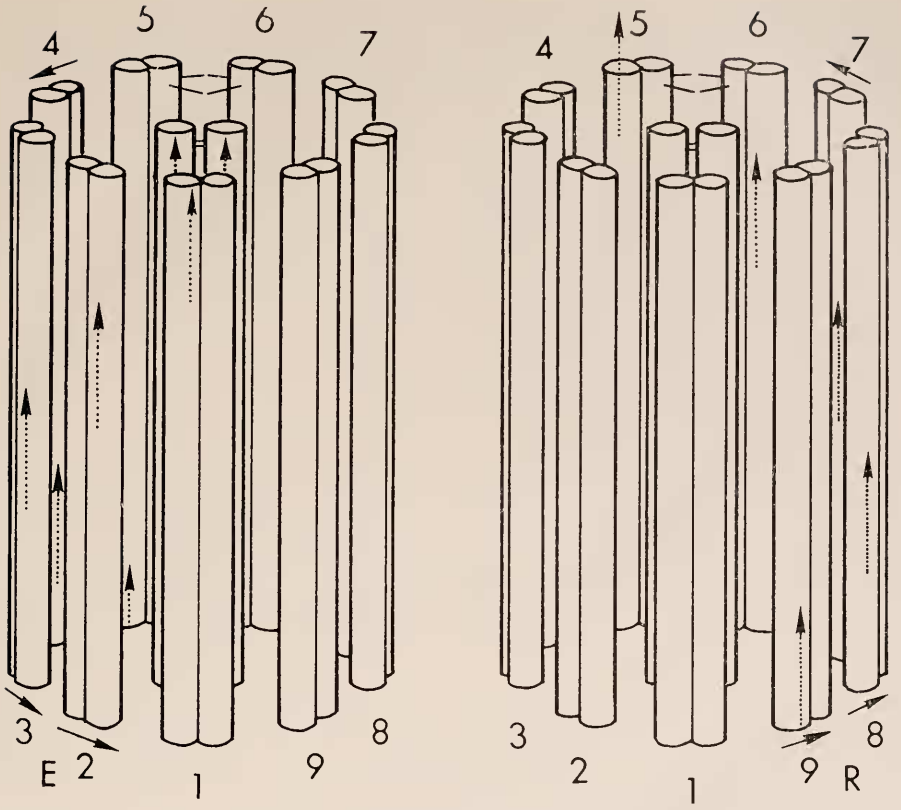


FIGURE 3. Propagation of waves out microtubules (dotted arrows) and direction of bending (solid, short arrows), for effective stroke (E) and recovery stroke (R), respectively. The dynein arms have been omitted, with the exception of one pair on doublet #5.

bridge connecting doublets #5 and #6 (Arrow E, Fig. 2). Since the central singlets of cilia of most forms do not originate at the basal plate, but at a point some distance (400 Å, at least) above it, basal bending of the cilium as a whole in this plane is not as much inhibited by the stiffened singlets as is the bending of the main shaft of the cilium on out to its tip. The significant factor here is not the distance, which is too little for a bending curve, but the fact that the singlets are not attached to the basal plate. Bending of the singlets in the lateral plane is not feasible because they reinforce each other in maintaining rigidity and cannot slide if they are fastened together. The effective stroke is, therefore, the bending, near the base, of an essentially stiff cilium, brought about by doubts #2, #3 and #4, in the direction of least resistance of the stiffened singlets, *i.e.*, at right angles to the plane connecting their central axes (Figs. 2 and 3E). The doublets which are not stimulated to bend (or singlets when not stimulated to stiffen) are relaxed and can be bent passively.

As the commutator continues to move and activates and then passes doublets

#5 and #6 (Fig. 3R), the central singlets gradually lose their stiffness and become limp progressively, from base to tip. The impulses progressively passing up doublets #2, #3 and #4 have also died out, successively, as they reached the tips of these microtubules. Doublets #5 and #6 are relatively ineffective in producing a lateral movement in the direction of their B-subtubules because (1) they are fastened together and dampen each other, (2) the central pair of singlets is still offering resistance to lateral bending, (3) the ciliary rootlets are so arranged as to inhibit lateral movement, and (4) the effective stroke has already carried the cilium over parallel to the cell surface. So such movement as is produced by attached doublets #5 and #6 will only cause the bent-over cilium to swing slightly out of the plane of the effective beat and somewhat to one side. In fact, doublets #5 and #6 bring the effective beat of the cilium to a complete halt. At the same time as these ineffective impulses terminate at the tips of #5 and #6, the stiffened state of the central singlets is terminated. Thus, when the commutator activates doublet #7 to bend in the direction away from its dynein arms, the recovery stroke is inaugurated. With relaxed central singlets, successive propagation of bending waves up doublets #7, #8 and #9 will produce half of a helical stroke (Figs. 2 and 3R). This is the recovery stroke, with the resultant of forces roughly in the direction opposite that of the effective stroke. This half helical wave will die out as the impulses reach the tips of these fibrils, ending the recovery stroke and completing one wavelength of ciliary beat. If the cilium is beating with little or no interruption, the commutator is again ready to activate the two central singlets and doublet #1 to begin a new beat.

This is, then, a situation where the half wavelength of the effective stroke is not identical with the half wavelength of the recovery stroke, since the two have different forms. What is identical is the length measured along the cilium for both the effective stroke and the recovery stroke, since this is the length of the cilium. (See also Párducz, 1953, 1954, 1961, 1967; Tamm, 1972).

It is assumed that the effective or power stroke carries the cilium from its resting position, whether this is upright or at any other angle, over to where it lies essentially parallel to the ciliated surface. The recovery stroke returns the cilium to its resting position. Therefore, the angle through which the cilium is moved may vary in different forms.

To summarize, ciliary motility consists of an effective stroke in one direction, brought about by doublets #2, #3 and #4, during a period when the central singlets are stiffened, and a recovery stroke, in the opposite direction, brought about by doublets #7, #8 and #9 while the central singlets relaxed. Doublet #1, and attached doublets #5 and #6, are essentially ineffective. It is the length of the axoneme that determines when the singlets are stiff and when relaxed, and when each lateral half doublet group is involved.

The most characteristic feature of ciliary motility is its biphasic nature, with the phases consisting of an effective stroke and a recovery stroke. The difference between these is, I believe, that the effective stroke involves the bending, at its base, of an essentially stiff organelle, while the recovery stroke involves the curvaceous return of a relaxed organelle. Any theory devised to account for ciliary movement must include, therefore, a mechanism for maintaining stiffness of the ciliary axoneme for half the duration of beat, and for achieving relaxation of this

stiffness during the other half of the beat. As indicated above, there is evidence that the central singlets become stiff upon stimulation, and I have postulated a relationship between the length of the axoneme and the duration of stiffness of the central singlets for an "ideal" cilium. So, while the simplest possible way to achieve an effective stroke and a recovery stroke of equal duration might be if the axonemal length were exactly equal to the length involved in $\frac{1}{2}\lambda$ of beat, it seems obvious that variations from this "ideal" condition may also have evolved. There are, for instance, very short cilia—probably of a length much shorter than that involved in a half wavelength. But, in such a very short cilium, bending through any angle up to 180° for the effective stroke might be accomplished by fewer than all the doublets of the first lateral half of the axoneme. Stimulation of the remaining doublets of this lateral half could contribute nothing more to the effective stroke because the cilium is already parallel to the substratum. Yet the recovery stroke cannot begin until the first of the doublets of the other lateral half is stimulated to bend in the opposite direction. The central singlets need be stiff only for the period of actual movement of the effective stroke, and this duration of stiffness would be correlated with their length. This suggests that the motility of cilia with lengths less than that postulated for the "ideal" cilium could be equally adequately explained by this same general theory. Perhaps, therefore, in ciliary motility, one should speak of the effective stroke, together with its "rest" periods, as occupying the *time* for a half cycle rather than a half wavelength of beat, and the recovery stroke, and its "rest" periods, as occurring during the period of the second half cycle of beat.

In ciliary motility, I visualize the bending waves, as such, actually moving out ciliary doublets #7, #8 and #9, whereas only basal bending takes place in doublets #2, #3 and #4, with the waves beyond this basal bending point dampened completely by the stiffened singlets. Each bending wave initiated and moving out any one doublet advances a distance equivalent to the contour length involved in 1/9th wavelength in the time of 1 9th cycle. An equal distance is traversed in the next 1/9th cycle. Meanwhile, a new bending wave is initiated in the next doublet and follows the same pattern.

Flagellar movement

Planar motility of 9 + 2 flagella. The foregoing leads to the problem of planar flagellar motility, the directional role of the central singlet microtubules therein, and whether there are stops at doublet #1 and at doublets #5 and #6 to permit reversal of direction of what would otherwise be an essentially uniform helical beat. In 9 + 2 flagella, and in the variants of these (9 + 9 + 2) which also possess a pair of central singlets, the continuing axonemal length (at least as long as required for a full wavelength of beat and usually very much longer) leads to a considerably different situation from that existing for cilia. Because of the increased length, there is more viscous resistance of the medium to be overcome. However, the continuing propagation of the bending waves out the doublets, and of the stiffening wave out the singlets, provides a more stable set of conditions than exists in the simple cilium. We assume for flagella a stiffening wave in the central singlets, which, as in cilia, will extend ahead of the bending wave in-

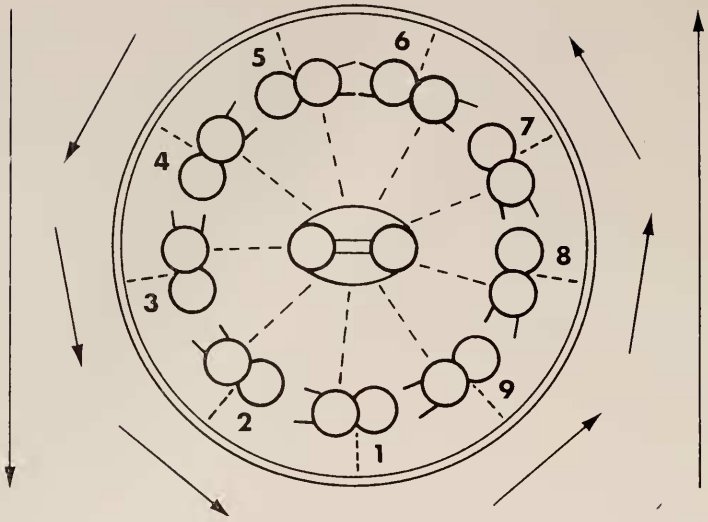


FIGURE 4. Diagram of cross-section of a 9 + 2 flagellum, showing direction of doublet bending for a planar wave.

augurated in the first doublet. The stiffness of the central singlets of a flagellum must persist in any given region for the duration of the active cycle of the wavelength.

As in cilia, basal activation of doublet #1 of the 9 + 2 flagellum will again be ineffective in producing bending, and for the same reasons. Activation of doublets #2, #3 and #4, successively, will result in the propagation of bending waves out the axoneme and produce its movement in one direction (Figs. 4 and 5). Resistance to lateral bending of doublets #5 and #6 is then encountered as they are activated, due to their attachment to each other, and to the continuing stiffness, *etc.*, of the central singlets. Now, however, because of the continuing length of the flagellar microtubules, the stiffening and bending waves do not terminate at the half-wavelength point, but continue moving out along the microtubules by which they are being propagated, for the full length of the axoneme. At the basal plate of the flagellar axoneme, 1/9 cycle after the activation of doublet #6, a bending wave is initiated in doublet #7, then successively in doublets #8 and #9, reversing the direction of the beat. With the singlets still stiff, these three doublets produce a planar return stroke. So, instead of an effective stroke and a recovery stroke, we have a complete planar wave propagated out the flagellum. The singlets may then relax, for the brief period between the activation of doublet #9, and the completion of the movement of the commutator around into position under doublet #1. Now, as the central singlets and doublets #1–#9, in order, are again successively activated at the base of the axoneme, a second wave, with the same characteristics, will be sent after the first. And, with the number present at any one time dependent upon the length of the axoneme, wave after wave will follow. With continuous flagellar motility, the distance apart of corresponding points of adjacent waves will be exactly one wavelength. The

stiffened central singlets of the flagellum have three functions: (1) to decrease the amplitude of the waves, (2) to prevent or inhibit lateral bending, and (3) to provide resistance and thereby facilitate unbending. If there is a complete inhibition of lateral bending, so the axoneme bends first toward one end and then toward the other of the axis through doublet #1 which bisects the space between #5 and #6, the flagellar movement would be completely planar (*i.e.*, forward and back on Figs. 4 and 5).

Now, since the period of non-lateral movement at doublets #5 and #6, in preparation for the reversal of beat, is of approximately twice the duration of that at doublet #1, the more abrupt reversal at the latter might create a slight backlash, which would be repeated at one-wavelength intervals along the flagellum. This backlash might create the rotational force that causes the spermatozoon to turn slowly on its axis, as described by Gray (1958) and others for bull sperm.. There is, then, a slight asymmetry in this otherwise symmetrical planar beat.

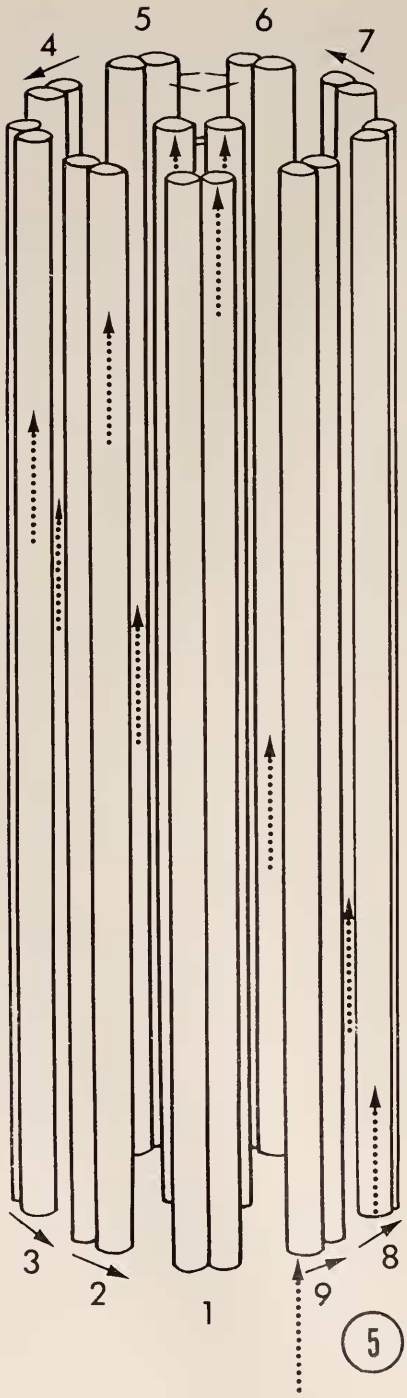
The complex bridges linking doublets #5 and #6 were described in the axoneme of echinoderm sperm tails by Afzelius (1959), in cilia of *Anodonta* by Gibbons (1961) and in gill cilia of *Elliptio* by Satir (1961). This bridge enables one to establish the axis of "bilaterality" which is correlated with the direction of the effective stroke of the cilium, which is related, also, to the orientation of the central singlets. It is not clear how widespread these #5–#6 bridges are in axonemes of spermatozoa. We have not seen them in any of the 9 + 0 or "9 + 1" axonemes of the acoels, rhabdocoels, triclads, or polyclads that we have examined. This suggests the possibility that they may be present only in cilia and in those sperm whose flagella have a planar beat.

The above view of planar flagellar motility is predicated on the supposition that the stiffening of the singlets provides sufficient resistance to lateral movement to inhibit completely actual bending of activated doublets #1, #5 and #6. Movement of the flagellum with bending at right angles to the plane through the centers of the two singlets must still be possible, however. Thus the bending of six doublets (#2, #3, #4 and #7, #8, #9), each in the direction away from its dynein arms, is responsible for this type of flagellar motility.

A less complete stiffening resistance to lateral bending may be the explanation for types of 9 + 2 axonemal motility intermediate between planar and helical. One must not ignore, however, the possible supplemental role in planar movement of components other than the singlets, present in some flagella, which are bilaterally disposed with respect to the plane of symmetry.

It is theoretically possible that under some circumstances, the activating stimuli to the central singlets of 9 + 2 flagellar axonemes are simply turned off or are non-functional. Under these circumstances these 9 + 2 axonemes would beat with a full helical beat.

9 + 0 axonemal motility. To account for axonemal motility of the 9 + 0 type, I suggest that a three-dimensional helical wave is generated by an impulse arising at the basal plate below doublet #1, and spreading around the plate in one direction, only, to produce propagated bending waves moving out each doublet, in serial order (Figs. 6, 7). Each doublet bends in a direction away from its dynein arms. If the commutator moves clockwise, the helical wave will be counterclockwise. A bending wave in any given doublet is 1/9 out of phase with the wave in either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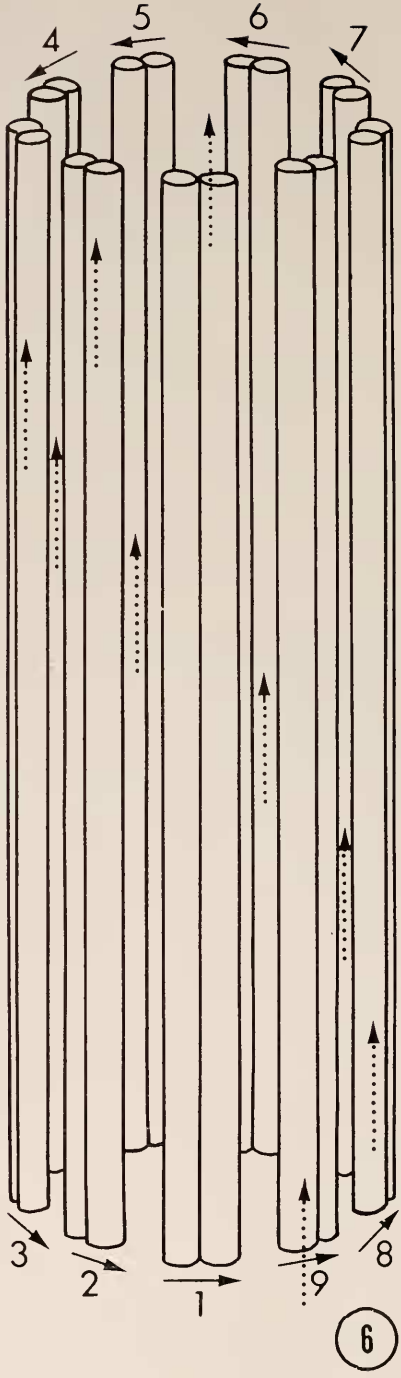
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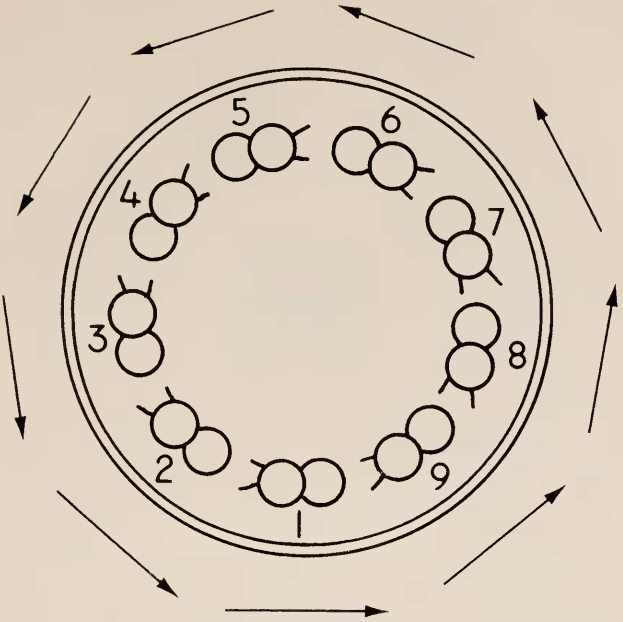

FIGURE 7. Diagram of cross-section of 9 + 0 flagellum, to indicate direction of successive bending of doublets to produce a wavelength of helical movement.

neighbor. Except for the directional involvement of the doublets (bending with the B-subtubule on the concave side) this view is essentially the same as that suggested by Bradfield (1955; pages 324–325) for the three-dimensional type of motility of 9 + 2 flagella (Fig. 9a). For these Bradfield included a statement that there was no conduction at all by the central fibrils. Of course, neither 9 + 0 nor "9 + 1" flagella had been discovered as early as 1955.

Logically, one wavelength of helical beat of a 9 + 0 sperm flagellum is produced by activation of doublets #1 through #9, and movement of the commutator back to #1 again, in preparation for the next wavelength of beat. As the first helical wave is propagated more distally along the flagellum, it is followed, without interruption, by successive waves of activation of doublets #1 through #9, the number of waves present at any one time depending on the length of the axoneme.

"9 + 1" axonemal motility. The "9 + 1" axoneme, with its 9 doublets and central core (Henley *et al.*, 1969) likewise moves with a helical wave (Fig. 8). It is assumed that the 9 doublets would be activated successively, in order, to produce one wavelength of helical beat. Successive waves would pass along the

FIGURE 5. Diagram of side view of one wavelength of a 9 + 2 flagellum to show propagation of waves out the microtubules to give planar movement. The dynein arms have been omitted, with the exception of one pair on doublet #5.

FIGURE 6. Diagram of side view of one wavelength of a 9 + 0 flagellum, to show propagation of waves out the doublets to give helical movement. The dynein arms of the doublets have been omitted.

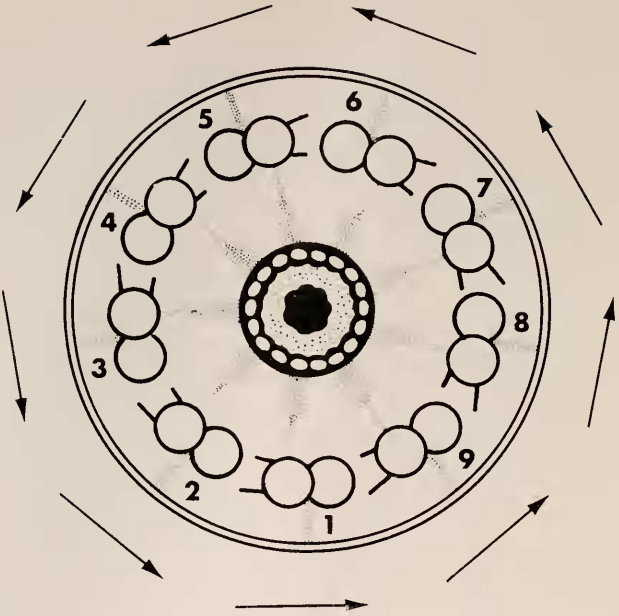

FIGURE 8. Diagram of a cross-section of a "9 + 1" flagellum to show central core and to indicate directions of successive bending of doublets to produce a wavelength of helical movement of reduced amplitude.

"9 + 1" axoneme, with the number present at any time depending upon axonemal length. Considerable resistance would be provided by the complex core because of its straightening tendencies (Henley, Costello, Thomas and Newton, 1969). However, since this resistance is nondirectional, as compared with that provided by a pair of central singlets, it may serve only to reduce the amplitude of beat, and perhaps contribute to unbending. It is of interest that some spermatozoa of triclad and polyclad flatworms have very long free flagella with "9 + 1" axonemes exceeding 200 microns in length, which are clearly observed to move with a helical beat.

DISCUSSION

Bradfield (1955) discussed possible mechanisms of flagellar and ciliary motility. He numbered the peripheral fibers 1 to 9, with #1 in the plane of symmetry. The direction of ciliary beat was assumed to be in the direction radially outward from fiber #1. For cilia, he assumed also that the impulse spreads around the ring in *both directions,* as well as to the central pair, which he considered to be more rapid conductors (Fig. 9a). For further details, see Bradfield's paper.

For flagella, Bradfield hypothesized that two-dimensional waves might be produced if the impulse spreads both directions around the base from #1 and dying out between #5 and #6 (or being reflected back) but *not* picked up and conducted rapidly along the flagellum by the central fibers in the manner postulated for the cilium. Bradfield suggested, also, that three-dimensional, corkscrew-like waves

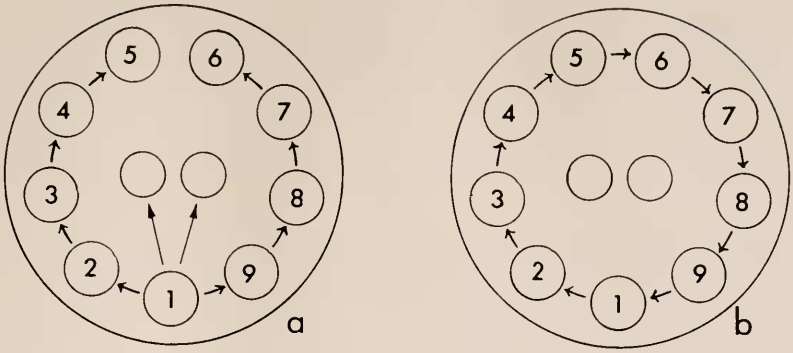

FIGURE 9. Bradfield's diagrams for (a) the ciliary beat and (b) the helical flagellar beat.

might be produced when the impulse originating under doublet #1 spread around the basal plate in one direction only, to fire off propagated contractions in each fiber in turn (Fig. 9b). Both these schemes for flagellar movement had the weakness of not suggesting any specific function for the central pair.

Minor modifications of Bradfield's theories by Gray (1955) and Sleigh (1960) present essentially the same difficulties. See also the reviews by Fawcett (1962), Sleigh (1962, 1968), Satir (1965b), and Holwill (1966, 1967).

Flagellar movement

There is a growing body of evidence from comparative studies by electron microscopy, suggesting that the central axonemal elements (whether they be the singlet microtubules of the 9 + 2 pattern or the core arrangement of "9 + 1" flagella of the sperm of certain flatworms) serve a stiffening function, acting in opposition to the bending of the doublet microtubules in effecting motility. The motile spermatozoa we have studied of those Turbellaria which have the 9 + 0 pattern (lacking any trace of central elements) have paired axonemes incorporated into two undulating membranes, each of which can move independently of the other, but which are normally integrated for effective motion. In some cases where the spermatozoa are very long and slender (notably the acoel flatworm *Polychoerus*) there is a keel-like central structure in the tail cytoplasm between the undulating membranes and their axonemes (Costello, unpublished data); this appears to be a centriole derivative, and to serve the stiffening function performed by the central elements of the axonemes in spermatozoa having such structures. The spermatozoon of the acoel *Childia* likewise has two 9 + 0 incorporated axonemes (Costello, Henley and Ault, 1969), but here there is no trace of the keel-like central structure. This may be correlated with the fact that the sperm of this form is much shorter and more compact than that of *Polychoerus* (50–60 microns *vs.* 400 microns in length) and the paired undulating membranes, into which the axonemes are incorporated, are considerably wider in the spermatozoon of *Childia*. Thus, the membranes themselves may impart the necessary opposing force of rigidity supplied by the central elements in sperm having other patterns of microtubule arrangement. The motile organelles of those flatworms with axonemes

having the "9 + 1" arrangement (see Henley *et al.*, 1969, for references) may occur either as very long free flagella, as in *Dugesia* and *Bdelloura* (Silveira and Porter, 1964) and *Mesostoma* (Henley *et al.*, 1969) or as paired axonemes closely applied to the body of the spermatozoon (as in *Stylochus*) and connected to it by very fine filamentous material (Thomas, 1971). In either case, however, the core appears to be considerably more rigid than the doublets in negatively stained "9 + 1" preparations.

Since 9 + 2, "9 + 1", and 9 + 0 axonemes of spermatozoa of Turbellaria were all found to be motile (Costello *et al.*, 1969; Henley *et al.*, 1969; Bedini and Papi, 1970; Hendelberg, 1970), it is clear that the doublet microtubules (or some part of them—as, for example, the dynein arms) are the significant components responsible for motility of these axonemes. Motile sperm with 9 + 0 axonemes are known in other groups also (for references, see Afzelius, 1970; Phillips, 1969, 1970a, 1970b). True 9 + 1 (9 doublets and one singlet) axonemes have been described for spermatozoa of certain scorpions (Hood, Watson, Deason and Benton, 1972).

It is worthy of note that the axonemes of spermatozoa of lower invertebrates all lack the coarse dense outer fibers characteristic of the flagella of many mammalian sperm. This, again, points to the doublet microtubules as the "simple common denominator" of axonemal motility.

I consider it highly significant that the surface cilia of these Turbellaria are of a single type, namely, 9 + 2, regardless of whether the sperm axonemes are 9 + 0, "9 + 1," or absent altogether. This suggests that ciliary motility differs from flagellar motility in that the ciliary stroke always requires directional alignment and that the central singlets within the axoneme determine or contribute to this.

Brokaw (1965) gave the parameters for the bending waves of the flagella of *Lytechinus*, *Ciona* and *Chaetopterus* spermatozoa. He considered these flagellar waves to be planar (pages 156, 160). The duration of time for the completion of one wavelength of beat was approximately 1/30 second, as determined from their beat frequency. They showed wavelengths of 22.6, 22 and 19.5 microns, respectively, and the contour lengths of the waves as measured along the flagella were 29.6, 30 and 25.5 microns, respectively. In *Chaetopterus* sperm, there were 1.25 waves for the overall length of the flagellum (31.875 microns). The actual length of the flagellum involved depends upon the wavelength, amplitude and exact form of the wave, and can be calculated by use of a cited formula (page 158). This means that for the half wavelength, the distance measured along the flagellum is 14.8, 15 and 12.75 microns, respectively. The surface cilia of the turbellarians I have studied were 12 to 15 microns in length and thus fall precisely within this range.

Harris (1961) considered the possibility that the rigidity of the cilium is provided by the two central fibrils, the peripheral ones being entirely contractile, but rejected the idea on the basis of a theoretical calculation that led him to believe they would have to possess a modulus of elasticity as high as that of a steel wire. I am not convinced of the validity of Harris' calculations. The total resistance leading to rigidity of the main body of the cilium during the effective stroke involves considerably more than that supplied by the central singlets. The non-activated doublets, matrix, membrane, periodic connectives between doublets and central

structures, connectives between doublets and membrane, nexin connectives, etc., must all offer some resistance both to propagated bending and to being bent backward by the medium. The stiffening supplied by the activated central singlets might be merely the final quantity that makes the effective stroke different from the recovery stroke.

Holwill (1966, page 750) has discussed this problem of compressive elements, involving these same components, and does not find impossible the likelihood of a high Young's modulus.

It is quite unnecessary to postulate, as Bradfield (1955), Sleigh (1962) and others have done, that turgor within the plasma membrane provides the stiffening. It was early shown that in glycerinated cilia, where the membranes and much of the matrix had been removed, the axonemes were still capable of movement. The recent experiments of Summers and Gibbons (1971) on isolated axonemes likewise show that turgor is not required.

Reversal of ciliary beat

In the very long (*ca.* 2000 microns) compound cilia of the comb-plates of *Mnemiopsis,* according to Afzelius (1961), the effective stroke is normally toward tubule #1, but in cilia of *Anodonta,* Gibbons (1961) has shown that the effective stroke is toward doublets #5 and #6. Since comb-plates of *Mnemiopsis* are capable of ciliary reversal and may, therefore, start their beat either toward doublet #1 or doublets #5 and #6, the question is, perhaps, academic.

However, in regard to reversal of direction of ciliary beat we may categorically state that if our view of the direction of bending of individual doublets is correct, reversal is not brought about by reversing the order of activation of the doublets. Instead, the reversed beat should start with activation at the opposite side (at doublets #5–6 instead of #1, for example), simultaneously sending impulses to the central singlets, and creating an effective stroke with doublets #7, #8 and #9, and the recovery stroke with doublets #2, #3 and #4. Hence the activating commutator would move in the same direction in the reversed and in the normal beat. In fact, if there were a brief refractory period after each ciliary stroke (to demarcate individual beats) there is no reason why the beat could not start with the activation of any one of the doublets. This might explain the various patterns of ciliary beating found by Sleigh (1968).

Ciliary versus *flagellar motility*

One of the significant facts emerging from these theoretical considerations is that a simple (non-compound) axonemal organelle, possessing stiffenable central singlets and of such length as to provide for only one-half wavelength or less must beat as a "typical" cilium, whereas a simple axonemal organelle of length equal to or greater than that needed to produce one wavelength at its characteristic amplitude and form would beat as a flagellum. The distinction between them is, therefore, length, in relation to wavelength, amplitude and form of beat. These constitute the basic differences between cilia and flagella.

If, under either experimental or "normal" conditions, the wavelength and amplitude relation of either type of organelle is sufficiently altered, a cilium might

be induced to beat as a flagellum, or a flagellum as a cilium. The production of a helical wave ("flagellar" wave) in the cilia of *Paramecium multimicronucleatum* by Kuznicki, Jahn and Fonseca (1970), brought about by treatment of the animals with 1.0% methyl cellulose for 3 to 24 hours, is clearly an example of just such an alteration of beat. Brokaw (1965, page 160) records, "Bending waves of greatly reduced radii of curvature [and therefore shorter wavelength] were observed when *Chaetopterus* spermatozoa were suspended in seawater solutions to which methyl cellulose had been added to increase the viscosity." Solutions with viscosities up to 300 centipoises were used in his experiments. Tamm (1972) has confirmed the observations that an increase in the viscosity of the medium can alter the mode of beat.

It is freely acknowledged, as Kuznicki *et al.* (1970) have pointed out, that all cilia, under all circumstances, do not beat with the back-and-forth movement ordinarily considered typical of these organelles. Similarly, all flagella, under all circumstances, do not beat with the helical undulations that are so much a part of all classical descriptions of flagellar motility. Students of protozoology and spermatology are well aware of the infinite variety of types of movement of these axonemal organelles. However, this theory is being presented as a simple basic view of axonemal mechanics and makes no attempt to explain the special features of unusual variations of motility other than 9 + 0 and "9 + 1" (9 + core) helical movement.

Sliding and bending mechanisms in motility of cilia and flagella

Summers and Gibbons (1971) presented evidence in favor of a sliding filament theory, with "ATP-induced shearing forces between outer tubules which, when resisted by the native structure, lead to localized sliding and generate an active bending movement" (page 3092). Isolated flagellar axonemes, briefly digested with trypsin, were found to actively disintegrate into individual tubules and groups on the addition of ATP. The disintegration resulted from active sliding between groups of doublets, together with a tendency for the partially disintegrated axoneme to coil into a helix (Summers and Gibbons, 1971).

The present paper places emphasis upon the bending activities which occur within the individual doublets and suggests that these may be of primary importance in the basic mechanism by which motility of cilia and flagella is accomplished. In normal motility, if the doublets remain attached to the basal plate, and if there is no change in their length (as observed for cilia by Satir, 1965a), the apparent sliding at the distal ends of the tubules might be incidental to whatever bending is taking place. Very little sliding movement appears to be possible in the intact cilium or flagellum, where the several types of connectives described above hold the component parts in relatively constant positions with respect to each other. Cilia or flagella which are just sufficiently macerated (with PTA) to destroy the connectives between doublets but not their attachments to the basal plate show the doublets splayed out in particular directions, bent, but not crawling over each other. In such isolated, partially macerated cilia, it has been possible in some cases to identify the doublets by number (Figs. 7 and 8 of the first paper of this series, Costello, 1973) and the directions of their bending correlated with their positions of attachment. Measurements of these doublets after moderate PTA maceration

show no increase in length as compared with those in an intact cilium. In addition, individual doublets can be isolated from their basal plates and will still show bending activities. However, our attention has been concentrated on the bending, looping, coiling, and spiraling activities of the doublet microtubules, since there was no direct evidence in this work to indicate that active sliding of doublets relative to each other was taking place. The information presented does not enable one to evaluate the relative merits of doublet interaction due to a sliding mechanism involving the dynein arms versus conformational changes in the subunit arrangements within the doublet microtubules themselves. Possibly both factors eventually may be shown to be involved.

Instead, the observations have been collated in an attempt to suggest the simple mechanics whereby a propagated unidirectional bending force acting in successive doublets, combined with stiffening activities in the central singlets (of 9 + 2 organelles) or central core (of "9 + 1" flagella), could give rise to either planar or helical movements of an axoneme, and, by interaction involving other features of the organelles, account for both ciliary and flagellar motility of various types.

Mechanisms for axonemal and microtubular motility may have evolved along pathways far different from those followed by mechanisms for contraction of striated muscle.

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Summary

1. A simple basic theory to account for the mechanics of ciliary and flagellar motility is proposed. It is based in large part on the conclusions resulting from observations made on axonemes of surface body cilia and of sperm flagella of a number of lower invertebrates after maceration and negative staining with phosphotungstate.

2. Ciliary motility consists of an effective stroke brought about by the successive bending of the three doublet microtubules of one lateral half of the axoneme while the central singlet microtubules are stiffened, and a recovery stroke, similarly brought about by the three doublets of the other lateral half, while the central singlets are relaxed. Bending of each doublet is in the direction away from its dynein arms. Doublet #1, and attached doublets #5 and #6, are rendered essentially ineffective by the stiffened singlets. The length of the axoneme in relation to wavelength determines when the singlets are stiff and when relaxed, and when each lateral half doublet group is involved.

3. Planar motility (of 9 + 2 flagella) consists of movement in one direction, brought about by successive bending of the three doublets of one lateral half of the axoneme, followed by movement in the other direction due to bending of the three doublets of the other lateral half, while the central singlets remain stiffened for the active period of the entire wavelength. Movement of doublets #1, #5, #6 is

inhibited by the stiffened singlets. It is the greater length of the flagellum that is chiefly responsible for the difference between flagellar and ciliary motility.

4. The helical motility of 9 + 0 and "9 + 1" axonemes is brought about by successive bending waves moving out all nine doublet microtubules, in sequential order, with no directional inhibition of any of them. The stiffened core of the "9 + 1" axoneme would be expected to decrease the amplitude of the helical beating in the latter type.

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